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Review

Fractionation of Polymers by Countercurrent Distribution

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The current situation in fractionation of polymers with liquid phase-pairs by countercurrent distribution (CCD) is briefly reviewed. The requirements for suitable phase-pairs are outlined and discussed. CCD methods can be deduced from the behavior of the polymers in nearly critical solvent systems. The fractionation occurs according to molecular weight and chemical composition. Three examples of such fractionations are given:

1. Polymethylmethacrylate is fractionated by CCD in a nearly critical, ternary phase-pair, modified by small amounts of a fourth solvent component.
2. A blend of polymethylmethacrylate and polyethylmethacrylate is separated in a separatory funnel by distribution in a ternary phase-pair.
3. A vinylchloride-vinylacetate-copolymer is fractionated according to its chemical composition by CCD in a nearly critical, quaternary phase-pair.

INTRODUCTION

The separations of low molecular weight substances in liquid phase-pairs in a separatory funnel or by countercurrent distribution (CCD) are well known and valuable methods.[1] In CCD the small separation effect caused by slight differences in the partition ratios of the compounds to be separated is amplified by repeating the distribution many times. With macromolecular substances, however, it is difficult to find suitable phase-pairs in which they dissolve and distribute into both phases. This is required for CCD if, as usually, the chemically similar components to be separated do not concentrate in opposite phases.

From a theoretical point of view the fractionation of polymers by distribution in liquid phases should be useful since, as Brönsted has demonstrated,[2] the logarithm of the Nernst partition ratio of macromolecules or colloid particles increases rapidly with molecular weight. As will be shown, the partition ratio is also strongly dependent on the chemical composition of both the solute and the solvent system. Therefore, CCD of polymers should offer good possibilities for the fractionation of various polymers according to molecular weight, chemical composition or even tacticity, provided suitable phase-pairs can be found.

In the literature several methods have been described which succeeded in isolating or fractionating macromolecules by CCD.[3] However, the method has not been widely applied and has been confined to a few types of polymers.

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At the Theodor Kocher Institute our group has looked in the past few years for phase-pairs suitable for the separation and fractionation of synthetic polymers and serum proteins and has studied their partition behavior. These investigations led to successful methods for the fractionation of polymers by CCD. Three examples will be given; the first a separation according to molecular weight, the others fractionations of chemically different species.

THEORY

According to *Nernst*,[4] a component dissolved and distributed in a liquid phase–pair has a definite concentration ratio, $K=\frac{c_u}{c_1}$, which is independent of the concentration for dilute solutions. K is called partition ratio. c_u and c_1 are the concentrations in the upper and lower phase, respectively.

Two components A and B can be separated by a few distribution steps, if their partition ratios K_A and K_B comply with the following two requirements:

$$\frac{K_A}{K_B}\sim 10-10^2 \qquad \text{and} \qquad K_A\cdot K_B\sim 1$$

At smaller values of $\frac{K_A}{K_B}$, successful separations can often be attained by multiple distribution steps or the so-called countercurrent extraction.[5]

Brönsted[2] found that the absolute value of the partition ratio increases rapidly with increasing molecular weight of macromolecules or colloid materials, in consequence of the fastly growing difference of the heat of solution in the two phases. He expressed the relation between the partition ratio and the molecular weight of homologous polymer series by

$$K=e^{\lambda\cdot M}$$

M is the molecular weight, and λ is a constant, relating a specific phase-pair to a dissolved polymer.

Schulz[6] published a more detailed expression of this dependence of the partition ratio:

$$\ln K=\frac{P(E_u-E_1)}{R\cdot T}$$

P is the polymerization degree, E_u and E_1 are the solution heats per monomer unit in the upper and lower phase, respectively. R is the gas constant and T the absolute temperature. The partition ratio of a particle changes with an increase or decrease of its molecular weight or a change of the composition of the two phases, caused either by a temperature shift or by an addition of small amounts of further solvent components.

As mentioned above, the separation of two different high molecular compounds by distribution in liquid phase-pairs can be carried out successfully, if they show a distinctly different partition behavior. But mostly, heterogeneous polymers have to be fractionated according to their chemical composition or molecular weight. The components i of such a polymer have all more or less one-sided partition in favor of the same phase, expressed by their individual partition ratios K_i. The mean partition

ratio $\bar{K}$ of the polymer is formed by these individual partition ratios and can be expressed according to *von Tavel:*[7]

$$\bar{K}=\frac{{}_i\Sigma\ \frac{K_i}{K_i+1}}{{}_i\Sigma\ \frac{1}{K_i+1}}$$

A CCD-fractionation can only be carried out successfully, if at least some of the components i are mainly dissolved in the opposite phase.

The base for calculation of theoretical distribution curves is the equation of the Craig' basic process:[8]

$$Y=\frac{1}{\sqrt{\frac{2\pi nvK}{(vK+1)^2}}}\cdot\exp\left[\frac{-x^2(vK+1)^2}{2nvK}\right]$$

Y: amount of polymer in the distribution unit x
n: number of distribution steps
K: partition ratio of a hypothetical homogeneous polymer
v: volume ratio of the upper and lower phase
x: number of the distribution unit. It is counted symmetrically with $x=0$ at the top M_d of the distribution curve.

$$M_d=\frac{nvK}{vK+1}$$

The comparison of an experimental distribution curve with this simple theoretical curve gives strong evidence of the homogenity of the original polymer.

SELECTION OF PHASE-PAIRS FOR THE DISTRIBUTION OF POLYMERS

The only known polymer, which dissolves in two mutually immiscible solvents is polyethyleneoxide. For other polymers the phase-pair has to be composed of at least three solvent components. To a good polymer solvent, two further liquids are added which mix with the polymer solvent but do not mix mutually. The polymer need not to be soluble in them. Usually, one of them will be more, the other one less polar than the solvent. It is useful to evaluate their suitability for polymer distribution using the well known Gibbs diagram. Systems of the type shown in Fig. 1 having a high miscibility gap and approximately horizontal tie lines, *i.e.*, with the plate point at the top of the binodal line, may be suitable for distribution of a polymer in both phases. Whether it will concentrate in one or the other phase depends on the content of polymer solvent in the phase and on the relative power of the phase forming liquids to precipitate the polymer from the solution.

Experience has shown that generally only nearly critical phase-pairs can dissolve enough polymer for fractionation (*i.e.* 0.5–2%). Each phase-pair of a ternary system corresponds to a tie line in the diagram. The solubility of the polymer decreases rapidly as the distance from the tie line to the plate point increases. An easily determined measure of this distance is the difference of the densities of the two coexistent phases or of their refractive indices. By one of these determinations a phase-pair can easily be defined exactly without complicated analysis of the phases. The volume proportion

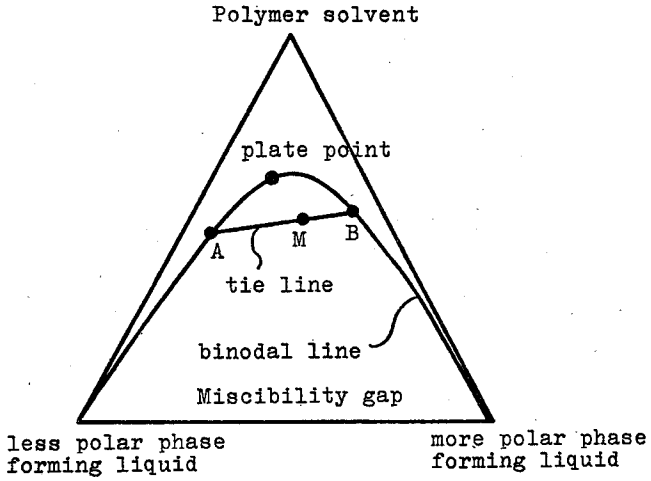

Fig. 1. Gibbs Phase diagram of a ternary system for polymer distribution.

of the phases B/A is the ratio of the distances M–A/M–B on the tie line.

The logarithm of the partition ratio of a polymer depends highly on the density difference of the phases. At the plate point it must be zero for every solute, whatever its constitution or molecular weight. With increasing density difference the absolute value of the logarithm of the partition ratio increases and the true value becomes negative, if the solute concentrates in the lower phase and positive if it accumulates in the upper phase. The higher the molecular weight of the solute, the more distinct is this shift. Observation showed that the increase is proportional to the density difference in the neighborhood of the plate point. This gives the opportunity to correct partition ratios so that they may be compared if measured in two phase-pairs of the same composition but different density differences.

Once a ternary phase-pair has been found that dissolves a polymer, it is very exceptional that its mean partition ratio lies between 0.2 and 5 as is desirable for a CCD–fractionation. Usually it concentrates totally in one or the other phase. By decreasing the density difference by adding polymer solvent it can be made to approach 1, but at the expense of selectivity. The better way to shift the partition ratios is to add a fourth solvent component, which should be able to increase the relative solubility of the polymer in the polymer-poor phase. In an alternative procedure, two ternary systems may be mixed in order to obtain an intermediate partition ratio. They should differ in only one solvent component and the polymer should accumulate in one of them in the more polar phase and in the other one in the less polar phase.

DISTRIBUTION EXPERIMENTS

Bieri[9] has found a suitable ternary system for the distribution of polymethylmethacrylate (PMMA). The nearly critical phase-pair consists of acetone (70% v/v) as polymer solvent, *n*-hexane (22%) and water (8%) as phase forming components. This phase-pair dissolves 1–2% (w/v) of PMMA at 20°C, when the density difference of the phases does not exceed 0.05 g/cm^3. PMMA dissolves mainly in the

lower more polar phase. The higher the molecular weight of PMMA, the larger the absolute value of the logarithm of its partition ratio will be. If small quantities of benzene, up to 1%, are added gradually, this absolute value decreases. It is 0 at 0.8% benzene and increases again rapidly if more benzene is added to approach asymptotically a value of 2–3. A plot of log $\overline{K}$ against the content of benzene displays a sigmoid curve (Fig. 2, right side). The higher the molecular weight, the higher the absolute value of log $\overline{K}$ and the steeper the angle at which the curve crosses the abscissa. This intersection, the point of equal distribution into both phases, is independent of molecular weight within experimental error.

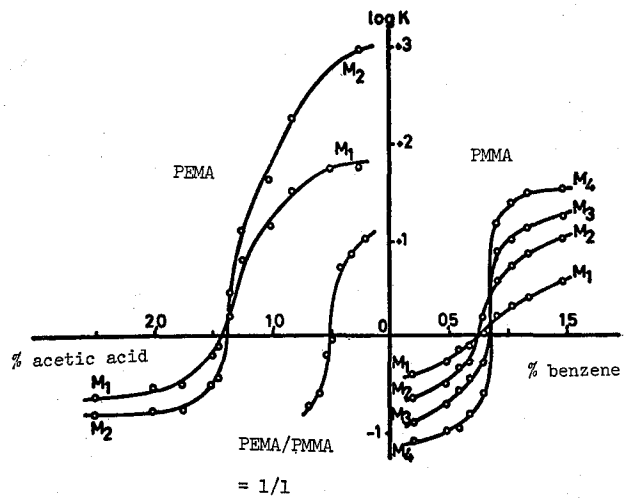

Fig. 2. Partition ratios of PMMA, PEMA, and a copolymer of PMMA/PEMA=1/1 in the phase-pair acetone/*n*-hexane/water to which benzene or acetic acid had been added. M_1, M_2,...: increasing molecular weight.[9]

In contrast to PMMA, polyethylmethacrylate (PEMA) concentrates in the nearly critical ternary phase-pair of acetone/*n*-hexane/water in the upper less polar phase. It can be shifted to the lower phase by adding gradually 1–2% acetic acid. The same sigmoid shape is obtained (Fig. 2, left side). Again the crossing of the abscissa is independent of the molecular weight. The point of equal distribution of the polymer, however, is characteristic of chemical composition. With copolymers in a given phase-pair it shifts steadily with the proportion of the monomers (Fig. 2 center). Even isotactic, syndiotactic and atactic PMMA have their points of equal distribution at distinctly different concentrations of benzene.

Copolymers of vinylchloride and vinylacetate (PVCAC) can be distributed in the same ternary phase-pairs of acetone/*n*-hexane/water, provided the vinylchloride content does not exceed 85 mole-%.[10] PVCAC shows a one-sided partition in favor of the upper phase. Its partition ratio can be varied between limiting values by a gradual substitution of either the solvent or one of the phase forming components:

acetone — tetrahydrofuran
n-hexane — *n*-heptane
water — acetic acid

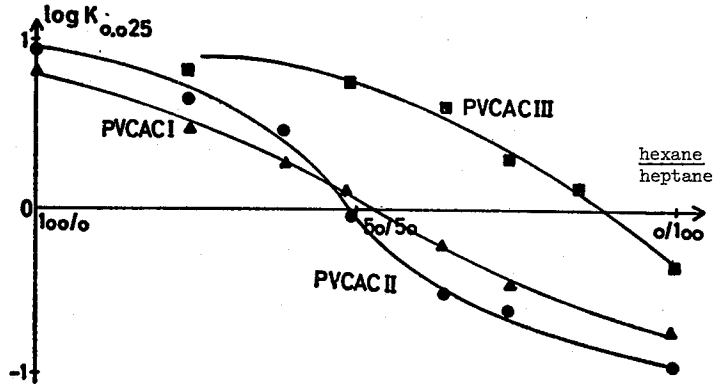

Fig. 3. Logarithm of partition ratios of different copolymers of vinylchloride and vinylacetate in ternary phase-pairs of acetone/water/*n*-hexane/*n*-heptane. PVCAC I and II: $\overline{M}_W$=40,000, molar vinylchloride content=62%, they differ only in their chemical heterogeneity. PVCAC III has a vinylchloride content of 84 mole-%.[10]

The partition ratio of PVCAC is also strongly dependent on its chemical composition (Fig. 3). PVCAC I, II, III are copolymers kindly supplied by Lonza AG, Fribourg, Switzerland. PVCAC I and II have approximately the same average molecular weight of 40,000 and the same average vinylchloride content of 62 mole-%. However, PVCAC II should be chemically more uniform than PVCAC I, because in its synthesis the proportion of vinylchloride/vinylacetate was kept constant, but not with PVCAC I. PVCAC III has a higher vinylchloride content of 84 mole-%. The sigmoid shape is not as pronounced as in the investigation of PMMA (Fig. 2). This is due to the lower molecular weight of the polymers and their chemical heterogeneity which result in the log $\overline{K}$-line crossing the abscissa at a smaller angle. The higher heterogeneity of PVCAC I compared to II is expressed in Fig. 3 by the flatter curve. The higher vinylchloride content of PVCAC III shifts the point of equal distribution considerably towards higher heptane content.

Hochuli[11] describes noncritical, quaternary phase-pairs for the distribution of polystyrene (PS). He shifts the partition of PS as desired in favour of the upper or lower phase by mixing the two noncritical phase-pairs A and B in suitable proportions:

A:	cyclohexane	38.5% v/v	B:	cyclohexane	61.8%
	nitromethane	35.5%		nitromethane	9.2%
	benzene	30.0%		nitrobenzene	29.0%

Mixtures of these two systems separate into phases of nearly equal volumes.

Reichert[12] fractionated copolymers of styrene and fumaric- or maleic acid diethyl ester, according to chemical composition or molecular weight by CCD in liquid phase-pairs consisting of up to nine solvent components.

FRACTIONATION OF POLYMERS BY CCD

The distribution behavior displayed in Figs. 2 and 3 suggest two procedures of

fractionation. With phase-pairs of constant composition, homologous polymer series should be fractionated according to molecular weight. On the other hand, a copolymer dissolved in one phase should be fractionated according to chemical composition by extraction with opposite phases of gradually changing composition. It is difficult to find procedures which fractionate either according to molecular weight or according to chemical composition. One of these properties, however, may prevail.

From the practical point of view the nearly critical phase-pairs have a great disadvantage. Their phases separate very slowly after they have been mixed in order to establish the distribution equilibrium. This is due to the small differences in density and surface tension. The time required for a distribution step in CCD is too long. We have constructed, therefore, at the Theodor Kocher Institute a distribution centrifuge with which the separation of the phases is accelerated. Its efficiency is slightly reduced compared to that of current CCD apparatus.[13]

FRACTIONATION OF PMMA ACCORDING TO MOLECULAR WEIGHT[14]

A PMMA sample with an average molecular weight of 170,000, kindly supplied by Lonza AG., was distributed in a phase-pair consisting of:

acetone	70% v/v
n-hexane	22%
water	8%

to which 0.6% benzene had been added.

At 21°C the density difference of the phases was 0.05 g/cm³. The distribution centrifuge was equipped with 60 tubes filled with 18 cm³ upper and 9 cm³ lower phase. The volume of the upper phase was made greater because the polymer distributes in this phase-pair in favor of the lower phase. The starting phase-pair contained 1.5% w/v PMMA and had a density difference of only 0.036 g/cm³ to increase the solubility. It was put in the first four tubes to increase the total polymer amount. To start the procedure from several tubes instead of one, impairs the selectivity slightly. After 300 automatic distribution steps, nearly all polymer had left the train and had been collected in about 200 fractions containing up to 10 mg PMMA. The residue of every tenth fraction was determined giving the distribution curve shown in Fig. 4. In other fractions the viscosity of the polymer was measured in chloroform. Several adjacent fractions had to be pooled in order to get sufficient material. From the viscosity values the molecular weight was calculated by the method of Schulz and Blaschke[15] with the constants $K=4.85\ 10^{-6}$ and $a=0.8$ from *Meyerhoff* and Schulz.[16]

The results are shown in Fig. 4. The polydisperse polymer has been separated into fractions of molecular weights ranging from below 50,000 to over 400,000. This is a proof of selectivity. The weight average molecular weight $\bar{M}_W$ compares well with that measured with the unfractionated PMMA. To what extent the molecular weight distribution found corresponds to the true distribution and to those found by other methods, *e.g.* gel permeation chromatography and ultracentrifugation must be shown by other investigations which are underway. So far this method compares favorably with other procedures.

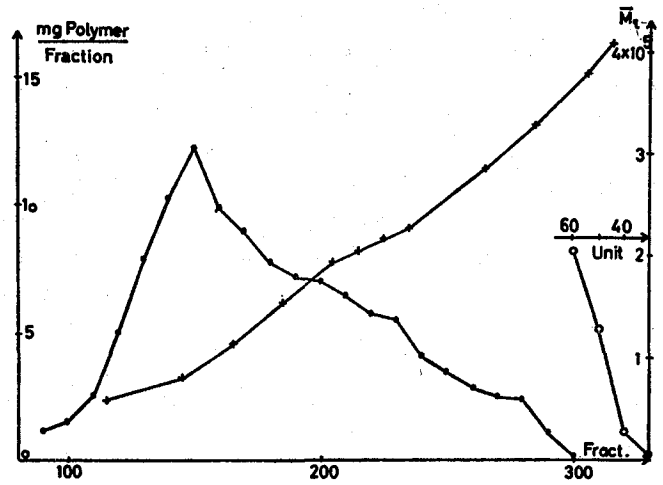

Fig. 4. CCD of PMMA. $\bar{M}_W = 170{,}000$. Distribution centrifuge, 60 tubes, 300 transfers in acetone/*n*-hexane/water/benzene phase-pair.[14]

SEPARATION OF A BLEND OF PMMA AND PEMA[9]

The great sensitivity of the distribution equilibrium to small chemical differences in the poymer can be demonstrated by the separation of a blend of PMMA and PEMA in a separatory funnel. As has been shown in Fig. 2 the two polymers concentrate in opposite phases of the acetone/*n*-hexane/water system mentioned above. A 1:1 blend of the two esters was distributed in this phase-pair in a separatory funnel. The

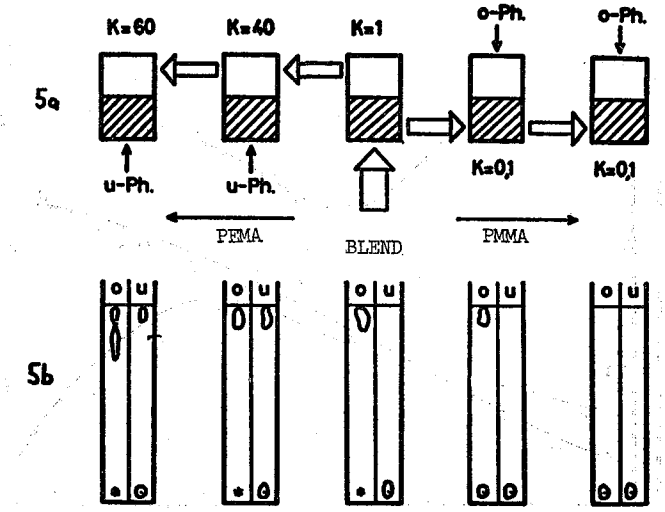

Fig. 5. 5a: Separation of PMMA and PEMA in a few distribution steps in a separatory funnel. Phase-pair of acetone/*n*-hexane/water. ⬆⬇ fresh phase added.

5b: TLC of the fractions, to the left upper, to the right lower phase. PMMA does not move on the TLC-plate; PEMA moves with the solvent front.[9]

phases were separated and equilibrated with fresh opposite phases of each. This process was repeated. From each phase a small sample was analysed before equilibration with new phase. The process is shown in Fig. 5 a. The $\overline{K}$-values are the partition ratios determined. Figure 5 b shows the TLC-analysis by the very useful method developed at the Institute for Chemical Research, Kyoto University.[17] As the blend contained only two chemically well defined, though polydisperse components, the separation was completed in a few distribution steps. Had the mixture been a series of gradually changing species the $\overline{K}$-values would have changed slowly with each transfer. This method provides a quick test for uniformity of polymer samples. It is facilitated when the concentrations can be determined by optical density measurements.

FRACTIONATION OF PVCAC ACCORDING TO CHEMICAL COMPOSITION[10]

PVCAC II was distributed in a nearly critical, quaternary phase-pair consisting of

acetone	63.5% (v/v)
n-hexane	11.3%
n-heptane	20.9%
water	4.3%

0.5% w/v PVCAC was dissolved in this phase-pair, having a density difference of 0.04 g/cm³ at 20°C. The CCD was carried out by 125 distribution steps on the centrifuge equipped with 60 tubes, the first six of them filled with polymer-containing phase-pair. As the PVCAC accumulates in this phase-pair in the lower phase, the volume ratio of the phases was kept at 2/1 in favor of the upper, mobile phase. The CCD was carried out as a basic Craig process[8] and stopped as soon as the first polymer

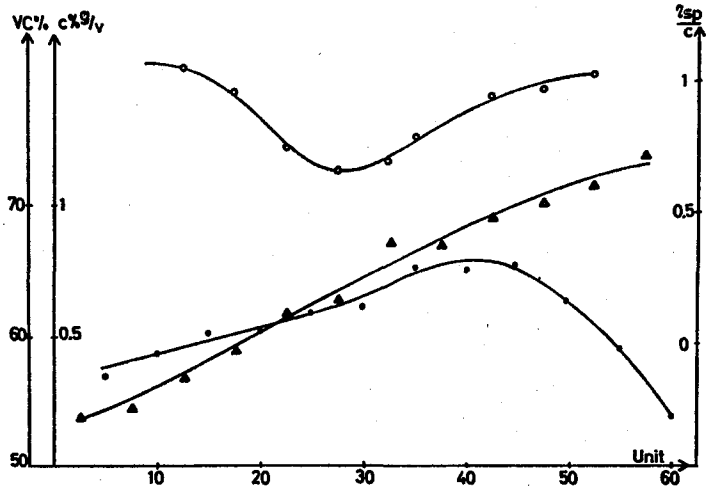

Fig. 6. CCD of PVCAC II. Distribution centrifuge. 60 tubes, 125 transfers in acetone/water/*n*-hexane/*n*-heptane phase-pair[10].

● weight distribution curve
△ vinylchloride content
○ specific viscosity

fractions left the train. So, the original PVCAC was separated into 60 fractions. The result of the fractionation is shown in Fig. 6. The residue of every fifth fraction was determined gravimetrically giving the weight distribution curve. The specific viscosities of the fractions show a minimum in the middle fractions. That is to be expected, if the polymer to be fractionated is too heterogeneous or the train used too short. The average chemical composition of the fractions was determined by their optical refraction. The vinylchloride content is rising regularly from 54 to 74 mole-%. It can be seen that the fractionation is very sensitive to chemical composition. However, fractionation according to molecular weight and monomer proportion occurs simultaneously, though in this case the chemical effect has prevailed.

CONCLUSION

The determination of molecular weight distributions or the distribution of other properties of polymers is always a tedious operation. None of the methods currently in use is completely satisfactory for precision determinations. New and better methods could still be useful.

CCD is a time and solvent consuming method like fractionation by *e.g.* precipitation and requires elaborate equipment, but it has shown interesting new possibilities especially for the fractionation of copolymers according to chemical composition. The method has proved to be useful for obtaining small samples which can be analysed and whose molecular weight and uniformity can be determined. With very heterogeneous material the selectivity is not the same over the whole range of properties but with prefractionated samples the determined distribution function should be correct.

A considerable advantage of CCD is the possibility of calculating the result. The comparison of calculated and experimental partition ratios offers an opportunity to control whether the experimental separation occurred according to the theoretical assumptions. It also can indicate the uniformity of unknown components in a fraction.

In the experimental procedure the theoretical assumptions can not be fully realized. The distributed polymer behaves like any other component of the system and influences the phase equilibrium. Therefore, the partition ratios are slightly dependent on polymer concentration and often a shift of phase volume in favor of the phase richer in polymer is observed. These effects can be reduced by lowering the quantity of polymer distributed and by increasing the phase density difference.

The fractionation becomes more effective as the molecular weight of the polymer rises. This is a special feature of this method, seldom encountered with other separation methods.

It is worth mentioning in this context that CCD of biopolymers leads also to interesting results. At the Theodor Kocher Institute the method was first applied to serum proteins, which could be fractionated at 0°C, in a phase-pair of diethylcarbitol/water/salts, *e.g.* magnesium sulfate or sodium glycerophosphate.[18]

Human γ-Globulin which is uniform in respect to molecular weight but chemically heterogeneous was separated into a large series of fractions, which had different partition ratios.[19] The 40 distribution steps, however, were not sufficient to separate individual antibodies.

Albertsson[20] has applied very interesting phase-pairs to the fractionation of proteins, nucleic acids and cell fragments. These phase-pairs consist of two incompatible polymers, *e.g.* polyethyleneglycol and dextran dissolved in water or diluted buffer solutions. If the molecular weight of these polymers is high enough, the phases may contain more than 95% water. They are, therefore, suitable for sensitive proteins and other biopolymers. In many cases it is difficult to separate the fractionated material from the phase forming polymers. Fractionated cell fragments, however, can easily be isolated by centrifugation.

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